

BIOLOGY AND HOST SPECIFICITY NOTES ON *STYPHLUS PENICILLUS* GYLLENHAL (COLEOPTERA: CURCULIONIDAE), EXAMINED AS A BIOLOGICAL CONTROL AGENT FOR *CRUPINA VULGARIS* IN THE UNITED STATES WITH REMARKS ON ITS HOST PLANT

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Abstract.—*Crupina vulgaris* Cassini appeared in the field in southern France (depending on rainfall) by late September, early April, and early and late May, respectively. Mines of the weevil were found on cotyledons three weeks after the appearance of seedlings during early October, and were present until early April of the following year. Eggs were deposited on the bases of cotyledons, under the epidermis, from late September to early October. There are three larval instars. Larvae kept at 6 to 18°C did not pupate but larvae kept at 15 to 28°C pupated, in the soil. Adults fed briefly during May and June before hiding under stones to estivate until early September to October, depending on weather conditions. To obtain an indication of the weevil's host range, various crop plants were grown in a field where *C. vulgaris* and *Styphlus penicillus* Gyllenhal occurred. Some, including artichoke, were attacked by *S. penicillus*, indicating that its host range is too broad to permit its use as biological control of *C. vulgaris*.

Key Words: *Styphlus penicillus*, *Crupina vulgaris*, weed, weevil, host range, biology

Crupina vulgaris Cassini is native to the Mediterranean region of Europe and has been reported from southern and eastern Europe, Turkey, the Middle East to Iran, Russia, and northern Africa (Bremer 1994). According to Couderc-Le Vaillant (1972), there are three species in the genus: *C. vulgaris* Cassini, *C. crupinastrum* (Moris) Arcangeli, and *C. intermedia* Brig et Cavill. The latter species is a hybrid between the first two; it is an aggressive competitor and can replace its parent species (Couderc-Le Vaillant and Roché 1993). *Crupina* is in the same tribe as the knapweeds, *Centaurea diffusa* J. B. A. P. Monnet de la Marck, *C. maculosa* J. B. A. P. Monnet de la Marck and *C. solstitialis* L. (Asteraceae: Cynareae). While seeds may remain viable for up to 32 months, the majority germinate the first year (Prather et al. 1991).

In Russia, *C. vulgaris* is a weed in semi-arid pastures. It was first discovered in the USA (Idaho) in 1968; it infests 63,500 acres in the Pacific northwest. Of these, 55,000 acres are rangelands of north central Idaho in the counties of Idaho, Lewis, and Clearwater. Washington State has an infestation of 480 acres in Chelan County. Oregon has an infestation of 8000 acres in Umatilla County, and California a 20-acre infestation in Sonoma County (Flanigan 1991). For Idaho, Belles et al. (1981) reported 8000 acres infested, while Callihan and Sanders (1994) reported 60,000 acres infested, an increase of 7.5 times in 13 years. There are indications of multiple introductions into the USA (Couderc-Le Vaillant and Roché 1993). Beside wind, water, and humans, deer, cows, horses, and pheas-

ants contribute to seed dispersal (Thill et al. 1986). Since it is not preferred by livestock, it has a competitive advantage and often replaces the native vegetation in annual grasslands similar to those invaded by yellow starthistle (Callihan and Sanders 1994). Prather et al. (1991) reported that no insect natural enemies or specific pathogens are available for biological control of this weed.

A project on biological control of *C. vulgaris* was started at the European Biological Control Laboratory, Montpellier, France, in 1992. Our survey in southern France showed that several natural enemies attack *C. vulgaris* in its homeland. One of these is *Styphlus penicillus* Gyllenhal (Coleoptera: Curculionidae). The adults of this weevil, which live under stones, moss, and in litter, were identified by E. Colonnelli, University of Rome, Italy. The larvae which mine the leaves were examined by N. J. Vandenberg, Systematic Entomology Laboratory (SEL), Washington, D.C., USA. This insect was reported on *Crepis (Barkhausia) taraxicifolia* D. C. (Asteraceae) and *Hedynois polymorpha* (P. Hervé) (Asteraceae) in southern France and in Germany (Hoffman 1958). No report could be found in the literature indicating *C. vulgaris* as a host plant for *S. penicillus*. The species was considered as a potential biological control agent for *Crupina vulgaris* because it attacks the seedlings at a time when they are small and are more vulnerable to competition from other plants. A field test carried out during September to December 1994 was conducted to determine the weevil's degree of host specificity and thus, whether or not the species could be employed against *C. vulgaris* in the United States.

MATERIAL AND METHODS

Biology.—Field and laboratory observations provided data on the bionomics of *S. penicillus*. To determine the number of larval instars, monthly samples of infested leaves were collected from December 1993 to March 1994 at St. Jean de Cuculles, 15

km north of Montpellier, France. The larvae were dissected from their mines and their head capsules were measured.

Since it was difficult to collect a sufficient number of adults to be used for experiments, we tried various methods for rearing larvae to the adult stage. A sample of 120 rosettes, with at least one mine per plant, was collected at St. Jean de Cuculles on 9 December 1993. Thirty were dissected to determine the number of larvae present in the mines. The remaining 90 rosettes were planted in 45 pots of 10 cm diameter (2/pot). Another 30 infested rosettes were collected at the same site on 13 December 1993 and were planted in the same kind of pots as above (2/pot). There were four sets of 15 pots, with 30 infested rosettes in each set. Two of the sets were placed in screen cages, one outdoors and the other in an unheated greenhouse. The third set was placed in an incubator with 8 h light, 15–18°C and 16 h dark, 6–7°C (cool incubator). The fourth set was placed in an incubator with 16 h light, 25–28°C and 8 h dark, 15–18°C (warm incubator). The aim of the experiment was to determine how the larvae could be reared to adults, to determine their damage to the plants, and survival of larvae. The rosettes were checked 20 January and 23 February 1994 and the number of leaves destroyed by the larvae and the number of larvae present on the plants were recorded. Adults that emerged from each set were recorded. The experiment was continued until 15 June 1994.

Infested leaves were field collected during March and April, when the larvae were mature. These leaves were placed in petri dishes on a layer of moist plaster of Paris and kept in the laboratory for rearing adults. Fourteen infested leaves of *Crepis taraxicifolia* were collected on 24 March and kept on moist soil for rearing adults for identification. The behavior of the adults reared from the plants was studied in the laboratory. Seven adults reared from *C. vulgaris* were caged in a glass cylinder (21 cm diameter and 20 cm high). A layer of sand

and a few small stones were placed on the bottom of the cage. *Crupina* plants were offered to the adults for feeding or oviposition. The cage was kept in the laboratory under ambient temperature and the weevils were examined frequently. The food plants were replaced as needed and the old plants were checked for oviposition.

To determine the density of a *S. penicillus* population in the field and its damage to *C. vulgaris*, eight patches with 7 to 27 rosettes each were selected (116 rosettes in total) at Montferrier, north of Montpellier, on 21 September, 1993; (in fact, at the beginning of the experiment, 17 patches with 158 plants were chosen, but eleven of them were destroyed by a tree cutting machine on 22 February). Each patch was in a circle of one meter diameter. The plants were checked monthly until 6 April 1994, and new plants that appeared in the field, the number of plants infested with *S. penicillus* larva, the number of cotyledons or real leaves that were destroyed, and the number of dead plants were recorded.

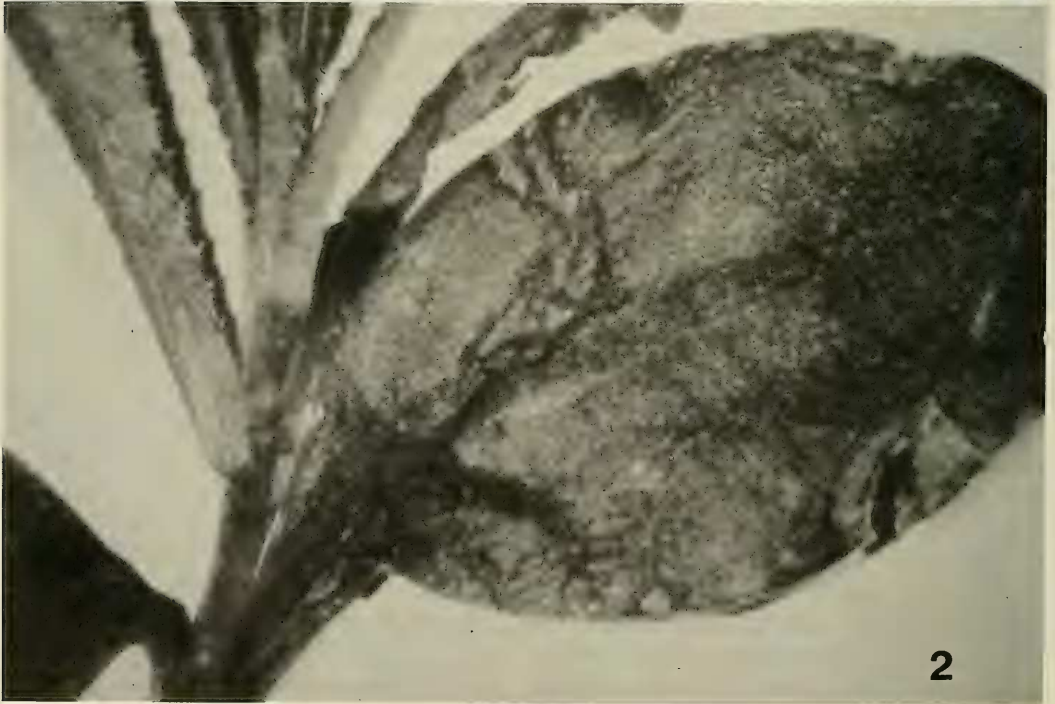
Field test to determine host specificity.—Seeds of the following test species were planted on 18 August, 1994: *Cynara scolymus* L. (artichoke), *Carthamus tinctorius* L. (safflower), *Helianthus annuus* L., *Lactuca sativa* L., *Tagetes erecta* L., *Calendula officinalis* L., *Cirsium pūcheri* (Torr.), *Zinnia elegans* Jacquin, *Aster chinensis* Nees, *Gazania splendens* Henderson, *Cichorium intybus* L., and *Crepis rubra* L. The seedlings were transplanted into small pots (10 cm diameter) on 10 September. The test plants were planted in a field at St. Jean de Cuculles, where *S. penicillus* and its host *C. vulgaris* were common, on 28 September and 15 October. They were placed among wild *C. vulgaris* seedlings at distances of 20–30 cm. Eight patches at a distance of 2–5 m were selected depending on the number of wild *C. vulgaris* rosettes present (5–30 rosettes/patch) (Fig. 1). The center of each patch was marked with a meter long iron bar, and the whole area was fenced with a red plastic net. Five patches were fenced

together as one plot and three other patches were fenced next to it at a distance of about 2.5 m. This resulted in two test plants of each of the 12 species being grown in each patch ($12 \times 2 \times 8 = 192$ plants). Seedlings of *C. vulgaris* had appeared in the field by September, 1994 and were common at this time. The objective of the test was to determine if *S. penicillus* would attack any of the test plants. All plants were checked visually for the presence of leaf miners (Fig. 1). Infested leaves were collected and examined under a stereomicroscope. Living larvae dissected from the mines were compared with the larvae of *S. penicillus* from *C. vulgaris* plants. Then they were preserved in alcohol and sent to SEL, Washington, D.C., for identification.

RESULT AND DISCUSSION

Biology.—*Crupina vulgaris* seedlings appeared in the field during the second half of September, depending on rainfall. The rosettes started bolting by 6 April, budding started by 10 May, and flowering by the end of May. Senescence started by the end of June and depended mainly on rainfall or soil moisture. *Styphlus penicillus* mines appeared in the cotyledons of *C. vulgaris* about four weeks after the seedlings appeared in the field. One to three larvae were found in infested cotyledons. After the larvae ate all of the leaf parenchyma, they moved to other cotyledons or leaves. During December to February there were larvae in different ages in their mines. The last larvae were found during early April.

The eggs were black, ovoid, 0.4 mm \times 0.8 mm and were deposited under the epidermis of the lower side, near the bases of cotyledons. Normally, one egg (rarely two) was deposited at each oviposition site. The emerging larvae mined toward the tips of the cotyledons, feeding on the upper and lower parenchyma. Measurements of the head capsules of 73 larvae showed that there were three larval instars. The head capsule of the L1 larva was 0.3 \times 0.4 mm ($n = 24$), L2 was 0.4 \times 0.5 mm ($n = 29$),



Figs. 1, 2. 1, Field experiment in St. Jean de Cuculles. 2, Damage to *Crupina vulgaris* leaf by *Styphlus penicillus* larva.

Table 1. Survival and feeding damage of *Styphlus penicillus* larvae on *Crupina vulgaris*, kept under four different conditions.

	Number <i>Styphlus</i> Larvae	Number Cotyledons Eaten	Number Leaves Eaten*	Number Dead Plants
20 January				
Outdoors	45	38/52	27	4/30
Greenhouse	34	16/48	23	6/30
Cold Incubator	36	39/48	34	6/30
Warm Incubator	16	42/50	22	5/30
23 February				
Outdoors	31	All except 4	53	13/30
Greenhouse	20	All	71	6/30
Cold Incubator	28	All except 1	71	7/30**
Warm Incubator	7	All except 3	32	5/30

* Total number of leaves was not recorded.

** Three and two larvae were resting in their mines on dry cotyledons.

and L3 was 0.5×0.6 mm ($n = 20$). The larvae were yellowish to milky white, with a dark brown head. They damaged the *C. vulgaris* seedlings and rosettes considerably (Fig. 2).

Thirty-two larvae were found in the 30 field collected rosettes and examined on 9 December, 1993. Living larvae were found in 24 rosettes and eight of these had two larvae/plant. All of the larvae were found in the cotyledons, except two, which had left them and penetrated into the leaves.

The best larval survival was observed in the cage outdoors and in the cold incubator. The highest plant mortality was in the cage outdoors and the highest feeding damage was in the cage in the greenhouse and in the cold incubator (Table 1). Ten adults emerged from the 15 pots kept in the greenhouse (30 March to 15 May) and five from 15 pots kept in the warm incubator (16 February to 4 March). No adults emerged from the 15 pots kept in the cold incubator by 15 June, when the experiment was terminated. It seems that the larvae need high temperatures to break their diapause. Twenty of the plants in the cage kept outdoors were dead by 10 March (for unknown reasons) and no adults emerged from them by 25 March, when the plants were destroyed.

Two of the ten larvae collected on 16

April and placed on plaster of Paris in a petri dish enclosed themselves in two tunnels, made of small pieces of the plaster of Paris. Two adults emerged from these tunnels three weeks later. However, 13 other larvae collected on 22 April 1993 and placed in the same kind of petri dish, in an incubator at 15–25°C, remained in their mines until they died. Some of the larvae in mines placed on moist soil and kept in the laboratory pupated in the soil. The majority of them remained in their mines on dry leaves for several weeks until they died. Eleven larvae were observed resting in their mines on dry leaves of potted *C. vulgaris* plants for several weeks until they died (they did not move to the fresh leaves).

Six adults emerged on 7 April 1994 from the sample of *Crepis taraxicifolia*. These also were identified by E. Colonnelli as *S. penicillus*. There were some differences in the color of the larvae and shape of the mines in *Crupina* and *Crepis*. The larvae in *Crupina* leaves were yellowish, but those in *Crepis* were milky white. In *Crupina* leaves, the miners mainly fed on the green parenchyma, while in *Crepis* leaves they mainly fed along the mid rib.

We observed minor feeding by the adults caged on *Crupina vulgaris* plants. They were relatively inactive; no copulation nor

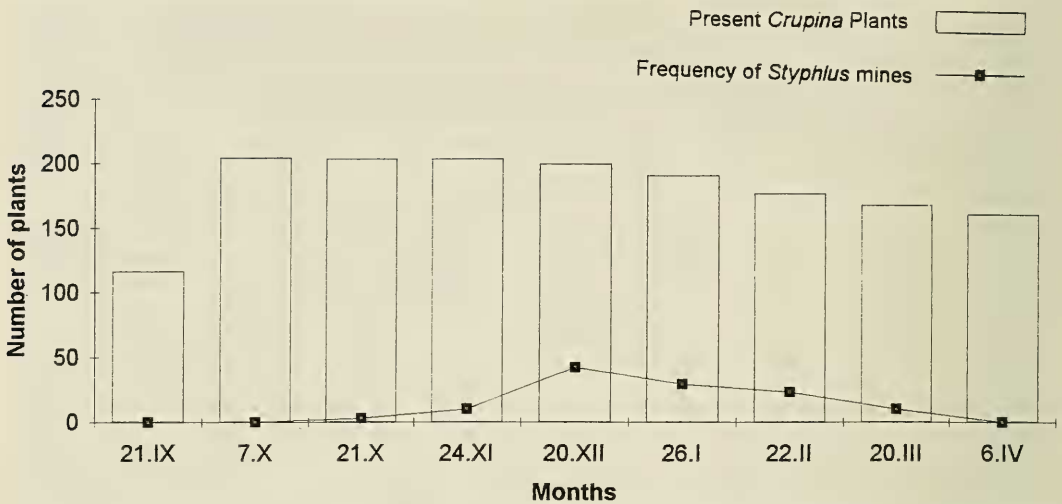


Fig. 3. Number of *Crupina vulgaris* plants and *Styphlus penicillus* mines on *Crupina* leaves in Montferrier during September 1993 to April 1994.

oviposition was observed. Five of the adults died during the period 12 April to 27 May 1994. The remaining two adults rested under stones until October 1994, when they started to feed on *Crupina* rosettes. The number of *C. vulgaris* seedlings increased from 116 on 21 September 1993, to 203 by 21 October 1993. Forty-three of these were dead by 6 April 1994 for unknown reasons. The plants were budding or flowering by mid May. Mines of *S. penicillus* appeared on cotyledons in October, increased during the second half of December, and decreased slowly until 6 April, when no more mines could be found on the plants (Fig. 3).

Field test to determine host specificity.—The first mine was observed on an artichoke leaf on 10 November 1994. Five more mines were found on artichoke leaves on 12 November. All of the mines contained living larvae, identical to *S. penicillus* larvae from *C. vulgaris*; D. M. Anderson and N. J. Vandenberg (SEL), who carefully examined these larvae and compared them with the *Styphlus penicillus* larvae, could not find any morphological differences between them. On 20 December, 1994, mines were also found on the leaves of the following test plants: *Cichorium intybus*, *Lactuca sativa*, *Crepis rubra*, *Cynara*

scolymus, and *Carthamus tinctorius*. Examination of these mines showed the presence of *Styphlus penicillus* larvae which could not be reared to adults. Since feeding was seen on these plants, it was apparent that this weevil has a wide host range and could attack crop and economically important plants. At this point, testing was discontinued and consideration of *S. penicillus* as a control agent of *C. vulgaris* ended.

Several other promising natural enemies have been reared from *C. vulgaris* in southern France and are under study. These are a pathogen of seedlings, *Ramularia* n. sp.; *Aceria balasi* Farkas (Acarina: Eriophyidae) which attacks flower buds; *Clytie ilunaris* Huebner (Lepidoptera: Noctuidae), a seed feeder; *Cochylini* sp. (Lepidoptera: Tortricidae), a defoliator; *Ornivalva pluteiformis* Staudinger (Lepidoptera: Gracillariidae), a seed feeder and *Metzneria aprilella* Herrich-Schäffer (Lepidoptera: Gelechiidae), a seed feeder.

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